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Laboratory Spawning Cues in 'Menidia beryllina' and 'M. peninsulae' (Pisces, Atherinidae) with Notes on Survival and Growth of Larvae at Different Salinities

(U.S.) Environmental Research Lab.
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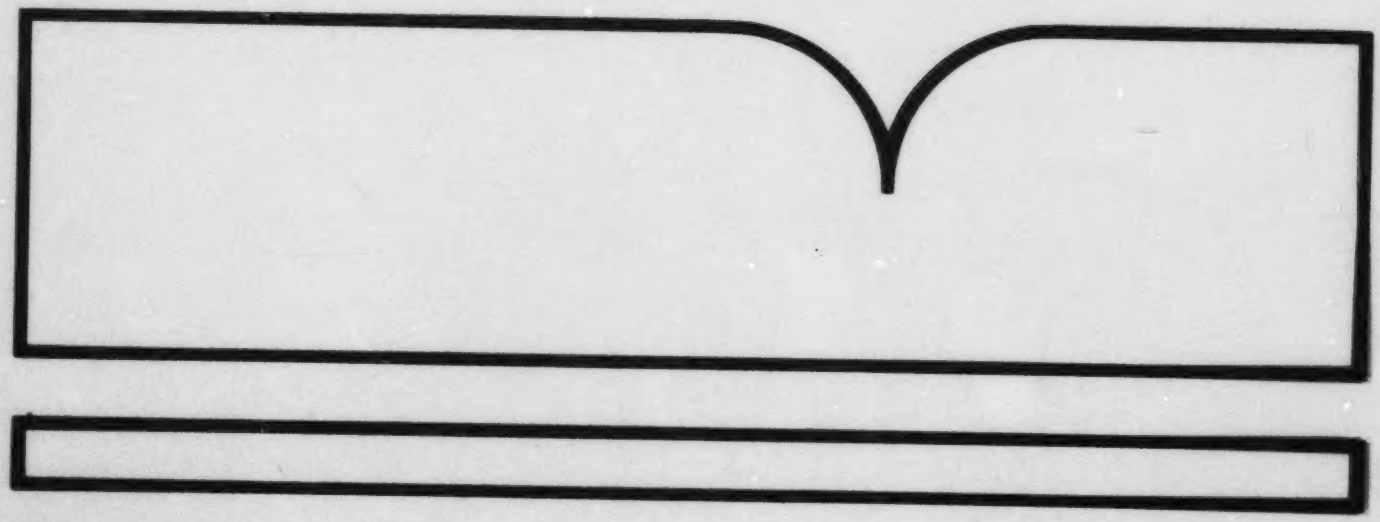

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16. ABSTRACT

Spawning patterns of inland silversides, Menidia beryllina, and tidewater silversides, Menidia peninsulae, were examined in the laboratory under several combinations of "tidal" and diel light cycle cues. M. beryllina showed a high frequency of spawning throughout the day when held under constant conditions (24L: 0D, current velocity 8 cm/sec) and when "tidal" and diel light cycles were presented singly or in combination. In contrast, M. peninsulae demonstrated a high frequency of spawning only when presented a combination of "tidal" and diel light cycle cues and spawned predominantly at night.

Menidia beryllina embryos were euryhaline. Hatching ranged from 73 to 78% at salinities of 5, 15 and 30°/oo. M. peninsulae embryos showed an inverse relationship between the percentage hatch and the incubation salinity, 90% at 5°/oo and only 65% at 30°/oo.

Survival and growth of larval M. beryllina from the day of hatching through 16 days old was optimal at 15°/oo. Although survival of M. peninsulae larvae was optimal at 30°/oo, no trend was apparent in growth of larvae held for 16 days at 5, 15 or 30°/oo salinity.

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Laboratory spawning cues in *Menidia beryllina* and *M. peninsulae* (Pisces, Atherinidae) with notes on survival and growth of larvae at different salinities*

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Keywords: Fishes, Photoperiod, Tidal cues, Reproduction

Synopsis

Spawning patterns of inland silversides, *Menidia beryllina*, and tidewater silversides, *Menidia peninsulae*, were examined in the laboratory under several combinations of 'tidal' and diel light cycle cues. *M. beryllina* showed a high frequency of spawning throughout the day when held under constant conditions (24L: OD, current velocity 8 cm sec^{-1}) and when 'tidal' and diel light cycles were presented singly or in combination. In contrast, *M. peninsulae* demonstrated a high frequency of spawning only when presented a combination of 'tidal' and diel light cycle cues and spawned predominantly at night. *Menidia beryllina* embryos were euryhaline. Hatching ranged from 73 to 78% at salinities of 5, 15 and 30‰. *M. peninsulae* embryos showed an inverse relationship between the percentage hatch and the incubation salinity, 90% at 5‰ and only 65% at 30‰. Survival and growth of larval *M. beryllina* from the day of hatching through 16 days old was optimal at 15‰. Although survival of *M. peninsulae* larvae was optimal at 30‰, no trend was apparent in growth of larvae held for 16 days at 5, 15 or 30‰ salinity.

Introduction

The tidewater silverside, *Menidia peninsulae*, recently recognized as a distinct species (Johnson 1975, Chernoff et al. 1981), was once considered conspecific with *Menidia beryllina* (Gosline 1949, Robbins 1969). These two atherinid fishes are often found in close proximity in estuaries along the southeastern coast of Florida and in the Gulf of Mexico (Echelle & Mosier 1982, Lucas 1982).

Despite sympatric occurrence and collection of *M. beryllina* and *M. peninsulae* in the same seine haul at four localities, two in Florida and two in Texas, Johnson (1975) observed that the species were discrete in these areas. Collections yielded a very low frequency of hybridization.

Although both species have been collected at salinities ranging from less than 5‰ to greater than 35‰, Johnson (1975) observed that *M. beryllina* generally occurred at salinities of 19‰ or less while *M. peninsulae* typically resided at salinities of 15‰ or greater. A similar trend was noted in coastal areas of Texas by Echelle & Mosier (1982).

Past confusion regarding the systematic status of each species (Chernoff et al. 1981) precluded acquisition of comparative data on the reproductive biology and early life stages of these fishes. Our laboratory study was conducted for several purposes: (1) examination of spawning by each species in response to diel light cycle and interrupted current

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velocity cues in the laboratory, (2) measurement of the salinity tolerance of embryos, and (3) determination of larval survival and growth patterns at low, intermediate and high salinities.

Materials and methods

Adult *Menidia beryllina* were collected during May-August approximately 2 km south of the Interstate-10 bridge along the shoreline at Bay Point in Blackwater Bay (Santa Rosa Co., Florida). The salinity ranged from 3 to 5‰ and water temperature from 23.7 to 26.3° C. Collections were made with a 10 m × 1m × 5 mm mesh bag seine. *M. beryllina* were carefully transferred by hand from the bag, which was not beached, to a 500 l aerated tank and transported to the laboratory. Similar procedures were used to collect *M. peninsulae* during April–September at Range Point, Santa Rosa Island (Escambia Co., Florida). Salinity ranged from 23 to 30‰ and water temperature from 22.0 to 24.4° C.

Laboratory spawning

Fifty individuals of each species, sex ratio 1:1, were stocked in two identical recirculating systems, each consisting of two circular fiberglass tanks, diameter 1.3 m, water depth 38 cm, and a filter-reservoir system. Experiments were conducted to determine the influence of environmental signals on the timing and frequency of spawning. Studies with *M. beryllina* were conducted at 5‰ (4–6‰) and 25.0° C (23.4–26.1° C), those with *M. peninsulae* at 26‰ (23–28‰) and 25.0° C (22.1–25.9° C). Because of space limitations, Experiments I, II and III were run sequentially. Before each experiment, newly collected fish were held for 3–6 days in the laboratory at respective test temperatures and salinities under a 13L:11D photoperiod. The seawater circulation pump ran continuously during pre-experiment holding.

A detailed description of the spawning system was provided in Middaugh & Hemmer (1984). In brief, seawater was pumped from a reservoir-filter system through each holding tank, producing a mean surface current velocity of 8 cm sec^{-1}. A gravity-flow drain returned water to the reservoir via a filter unit containing layers of crushed oyster shell, activated charcoal, coarse pebbles and aquarium filter fiber. The spawning tanks were syphoned clean and the activated charcoal changed weekly. Approximately 20% of the water in each system was replaced with salinity-adjusted seawater each week.

Pairs of tanks were isolated by light-tight curtains. Fluorescent lamps centered above each tank provided a light intensity of approximately 175–200 lux during daytime. Periodic interruptions in current velocity from 8 to 0 cm sec^{-1} were achieved by turning off the seawater circulation pumps for 1 hour twice daily. These interruptions were designed to simulate decreases in current velocity during slack high (or low) tides in the field. Current velocity decreased from 8 to 0 cm sec^{-1} within 3 to 5 minutes after turning off the circulation pump and rapidly returned to 8 cm sec^{-1} when the pump was restarted. A synopsis of cueing regimes utilized in experiments with *M. beryllina* and *M. peninsulae* is presented in Table 1.

A spawning substrate made of polyester aquarium filter-fiber, approximately 15 cm long × 10 cm wide × 10 cm thick was suspended just below the water's surface and in contact with the side of each holding tank. Substrates were checked for eggs between 0800 and 1200 h daily. If spawning occurred, the developmental stages of two randomly selected groups of at least 20 eggs were determined microscopically. The classification scheme of Lagler et al. (1977) was used to identify developmental stages. The time of spawning was estimated by comparing developmental stages with stages of known age eggs incubated at similar water temperatures.

A summary of survival, wet weight, standard length (SL); and the ratio of horizontal distance (HD) between the origin of the first spinous dorsal and the origin of the anal fin, and standard length (HD/SL) for brood stocks used in Experiments I, II and III is provided in Table 2. Our measurements of HD/SL agree well with mean values of 0.054 for *M. beryllina* and 0.090 for *M. peninsulae* reported by Chernoff et al. (1981).

Table 1. Synopsis of laboratory spawning experiments with *M. beryllina* and *M. peninsulae* during sequential 14–28 day periods of three experiments.

Experiment I		Experiment II		Experiment III	
Duration (days)	Environmental conditions	Duration (days)	Environmental conditions	Duration (days)	Environmental conditions
14	24L: OD Constant current of 8 cm sec^{-1}	14	24L: OD Constant current of 8 cm sec^{-1}	14	24L: OD Constant current of 8 cm sec^{-1}
14	13L: 11D Constant current of 8 cm sec^{-1}	14	24L: OD 0 cm sec^{-1} at 0600–0700, 1800–1900	21–28	13L: 11D 0 cm sec^{-1} at 1200–1300, 2400–0100
14	13L: 11D 0 cm sec^{-1} at 0600–0700, 1800–1900	14	13L: 11D 0 cm sec^{-1} at 0600–0700, 1800–1900	14	24L: OD Constant current of 8 cm sec^{-1}
14	24L: OD Constant current of 8 cm sec^{-1}	14	24L: OD Constant current of 8 cm sec^{-1}	–	–

Table 2. Summary of survival and morphometric data for *M. beryllina* and *M. peninsulae* adult spawners used in Experiments I, II and III.

Species and variable	Experiment I		Experiment II		Experiment III	
	Male	Female	Male	Female	Male	Female
Menidia beryllina						
% Survival	59	73	38	69	33	39
$\bar{X}$ wet weight (g)	1.4	1.9	1.6	1.9	2.4	2.0
$\bar{X}$ SL (mm)	49.2	55.3	51.8	54.8	57.1	56.3
$\bar{X}$ ratio HD/SL*	0.05	0.06	0.05	0.06	0.05	0.06
HD/SL (range)	(0.03–0.06)	(0.04–0.08)	(0.04–0.07)	(0.04–0.07)	(0.04–0.07)	(0.04–0.08)
Menidia peninsulae						
% Survival	70	48	80	52	73	60
$\bar{X}$ wet wt. (g)	2.9	2.7	2.7	2.4	4.0	4.8
$\bar{X}$ SL (mm)	59.9	58.5	59.7	58.3	70.4	72.8
$\bar{X}$ ratio HD/SL*	0.09	0.09	0.09	0.09	0.09	0.09
HD/SL (range)	(0.07–0.11)	(0.07–0.11)	(0.07–0.11)	(0.06–0.11)	(0.08–0.11)	(0.07–0.11)

* $\bar{X}$ ratio HD/SL-ratio of horizontal distance between the origin of the first spinous dorsal fin and origin of the anal fin divided by the standard length.

Survival of embryos

Tests were conducted to determine the effect of three salinities (5, 15, 30‰) on the survival of developing embryos until hatching. Thirty fertilized eggs of *M. beryllina* (stages 6 to 8, Lagler et al. 1977) which had been spawned at 5‰ and 25 ± 1° C were simultaneously transferred to each of four 250 ml beakers at 5, 15 or 30‰. Beakers were maintained at 25 ± 1° C and daily observations made for embryo survival and hatching. Dead individuals were removed and counted. Similar procedures were utilized in tests with *M. peninsulae* except that embryos (stages 6 to 8) were spawned at 26‰ and 25 ± 1° C prior to transfer to 5, 15 or 30‰.

Larval survival and growth

Survival and growth of *M. beryllina* and *M. peninsulae* were determined during 16 day tests in which each species was cultured at 5, 15 and 30‰. Fertilized eggs (stages 6 to 8) were teased from spawning substrates maintained at 5‰ for *M. beryllina* or 26‰ for *M. peninsulae*, and 25 ± 1° C. They were transferred to 4 l aquaria containing 3 l of filtered seawater at 5, 15 and 30‰ and held at 25 ± 1° C until hatching. On the day of hatching (DOH), 100 individuals from each salinity regime were transferred to two 4 l aquaria containing filtered seawater at the incubation temperature and salinity. Larvae were fed rotifiers, *Brachionus* sp. at a concentration of 5000 l^{-1} on the DOH, and daily thereafter until 7 days after hatching. On days 8 and 9 both species were fed *Artemia* nauplii at a concentration of 5000 l^{-1}, once daily. On day 11, all fish were transferrd to 20 l aquaria for the duration of the 16 day grow-out period and fed *Artemia* at a concentration of 5000 l^{-1}, twice daily. In all cases large bore glass pipettes (3 mm I.D.) were used to transfer larvae. Use of dipnets results in mass mortalities shortly after handling of larval *Menidia*. Approximately 10% of the volume of culture aquaria was exchanged every other day using salinity and temperature-adjusted filtered seawater.

The first aquarium in each pair at respective salinities of 5, 15 or 30‰ was used to monitor daily survival. Ten fish were removed from the second aquarium on DOH, 4, 8, 12 and 16 days after hatching to determine total (TL) and standard lengths (SL), and wet and dry weights. Total and standard lengths were measured using a stage micrometer. Wet weights of individuals were determined (to the nearest 0.01 mg) after gently blotting. Individuals were then placed on a tared weighing pan, dried for 24 hours at 90° C, cooled for two hours in a desiccator and reweighed.

Results

Laboratory spawning

The frequency and timing of spawning by *M. beryllina* and *M. peninsulae* differed markedly when the two species were held under similar environmental conditions (refer to Table 1). In Experiment I during the first 14 days under constant conditions (24L:OD, current velocity 8 cm sec^{-1}) *M. beryllina* spawned frequently and throughout the day. In contrast, *M. peninsulae* spawned infrequently. There was no evidence of a daily spawning rhythm in either species (Fig. 1a). Introduction of a 13L:11D photoperiod with a constant current velocity (Fig. 1b) elicited a distinct spawning response in *M. beryllina* with most of the reproductive activity occurring between 1900 and 2300 h. *M. peninsulae* continued to spawn infrequently. Insertion of twice daily interruptions in current velocity (Fig. 1c) had little influence on the spawning pattern of *M. beryllina*; reproductive activity continued to occur most often during night. In contrast, *M. peninsulae* showed a marked increase in the frequency of spawning and there was an obvious nocturnal spawning cycle with most reproductive activity taking place between 1800 and 2300 h (Fig. 1c). After constant conditions were resumed, the frequency and timing of spawning in *M. beryllina* remained similar to the previous 14 day period (Fig. 1c, d). However, the frequency of spawning by *M. peninsulae* declined and spawning was arrhythmic (Fig. 1d).

In Experiment II, the timing and frequency of spawning by *M. beryllina* and *M. peninsulae* during the first 14 days (Fig. 2a) was similar to that observed in Experiment I (Fig. 1a). In contrast, *M. beryllina* provided with twice daily interruptions of current velocity (Fig. 2b) generally spawned between 0500 and 1200 h apparently in response to the interruption in current velocity from 0600 to 0700 h. The trend of low frequency arrhythmic spawning continued in *M. peninsulae* (Fig. 2b). The combined influence of a diel light cycle and interrupted current velocity (Fig. 2c) resulted in asynchronous spawning in *M. beryllina*, however, *M. peninsulae* spawned primarily between 1800 and 2300 h, and the frequency of spawning increased, a pattern similar to that observed in Experiment I (Fig. 1c). Introduction of constant conditions for the final 14 days of Experiment II resulted in asynchronous spawning in both species (Fig. 2d).

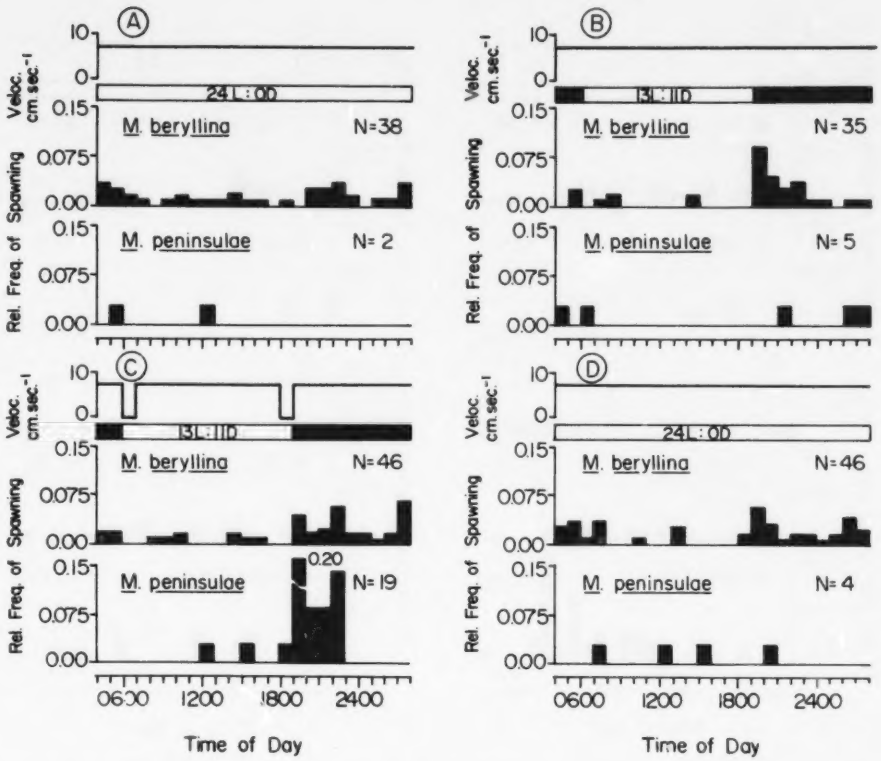

Fig. 1. Relative frequency of spawning by *M. beryllina* and *M. peninsulae* during respective two week periods in Experiment I: (A) constant conditions, (B) constant current velocity, 13L: 11D, (C) interrupted current velocity twice daily, 13L: 11D, (D) constant conditions. Refer to Table 1 for a synopsis of environmental spawning cues.

Because each set of environmental cues could have influenced the response of fish to subsequent cue combinations, we were unable to assume independence of spawning for each of the 14-day periods of Experiments I and II. Nevertheless, since each species was subjected to identical parameters during each experiment, we were able to make between-species comparisons of spawning within Experiments I and II. Chi-square analyses (Marascuilo & McSweeny 1977) revealed a significant difference in the frequency of spawning for *M. beryllina* and *M. peninsulae* in response to environmental cues in Experiment I, $\chi^2 = 15.24$, df = 5, $P<0.05$, and in Experiment II, $\chi^2 = 21.13$, df = 5, $P<0.05$.

In Experiment III, our goal was to determine the response of each species to diel light cycle and interrupted current velocity cues when the two events were presented in concert, but as discrete signals. *M. beryllina* and *M. peninsulae* were maintained under similar diel light cycle and interrupted current velocity cues (Table 1). However, in contrast to Experiments I and II, the interrupted current velocity cue occurred midway through the light and dark phases, rather than just after lights on and lights off.

Under constant conditions, *M. beryllina* spawned frequently and throughout the day. In contrast, *M. peninsulae* showed a very low frequency of spawning (Fig. 3a). Introduction of diel light cycle and interrupted current velocity cues resulted in continued frequent spawning by *M. beryllina* with most activity just after lights on or prior to lights off. *M. peninsulae* spawned during

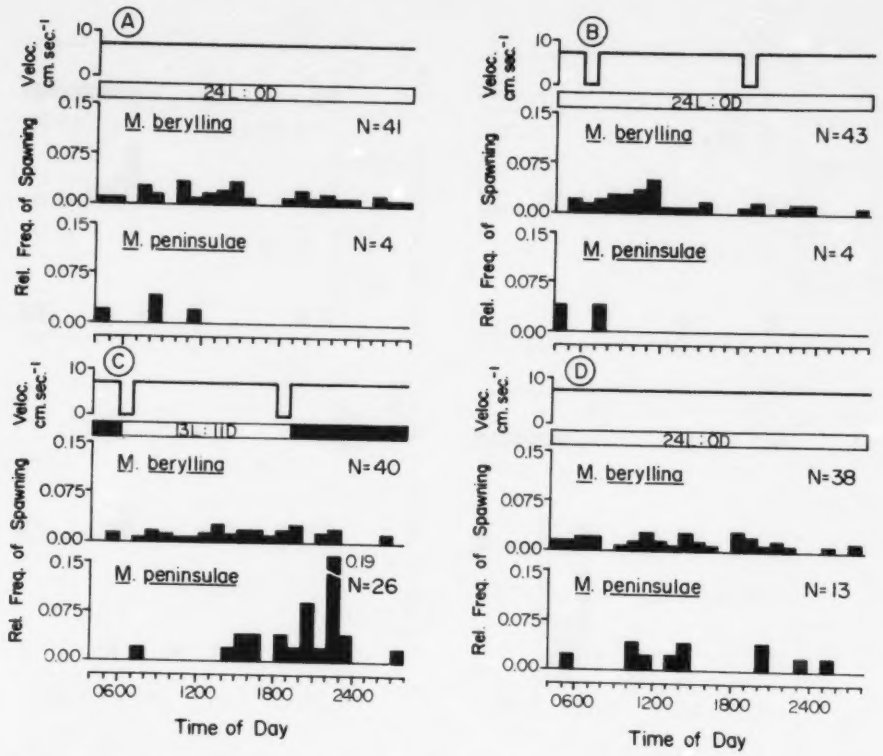

Fig. 2. Relative frequency of spawning by *M. beryllina* and *M. peninsulae* during respective two week periods in Experiment II: (A) constant conditions, (B) interrupted current velocity twice daily, 24L: OD, (C) interrupted current velocity twice daily, 13L: 11D, (D) constant conditions. Refer to Table 1 for a synopsis of environmental spawning cues.

darkness, with a peak in activity during the period of interrupted current velocity between 2400 and 0100 h (Fig. 3b). Return of fish to constant conditions had no apparent influence on *M. beryllina*, spawning continued to occur throughout the day. *M. peninsulae* spawned primarily during darkness, between 2300 and 0400 h (Fig. 3c). Chi-square analyses were not conducted on spawning frequencies for *M. beryllina* and *M. peninsulae* in Experiment III because the duration of intervals was dissimilar (Table 1).

Embryo survival

M. beryllina embryos hatched 6 to 8 days after fertilization when maintained at 5, 15 or 30‰ and 25 ± 1° C. The mean hatching for replicates ranged from 73 to 78%. *M. peninsulae* showed similar rates of development, 6 to 8 days, but demonstrated a trend of decreased hatching as salinity increased (Fig. 4).

A one-way ANOVA and post-hoc Student-Newman-Keuls procedures revealed no significant differences ($P > 0.05$) in hatching of *M. beryllina* embryos maintained at 5, 15 and 30‰ (SAS User's Guide 1982). However, the number of hatching *M. peninsulae* was significantly different at each salinity ($P < 0.05$).

Between species comparisons at each salinity revealed significant differences in hatching of *M. beryllina* and *M. peninsulae* held at 5 and 30‰ ($P < 0.05$) but not at the intermediate salinity of 15‰.

Larval survival and growth

M. beryllina larvae showed optimal survival and

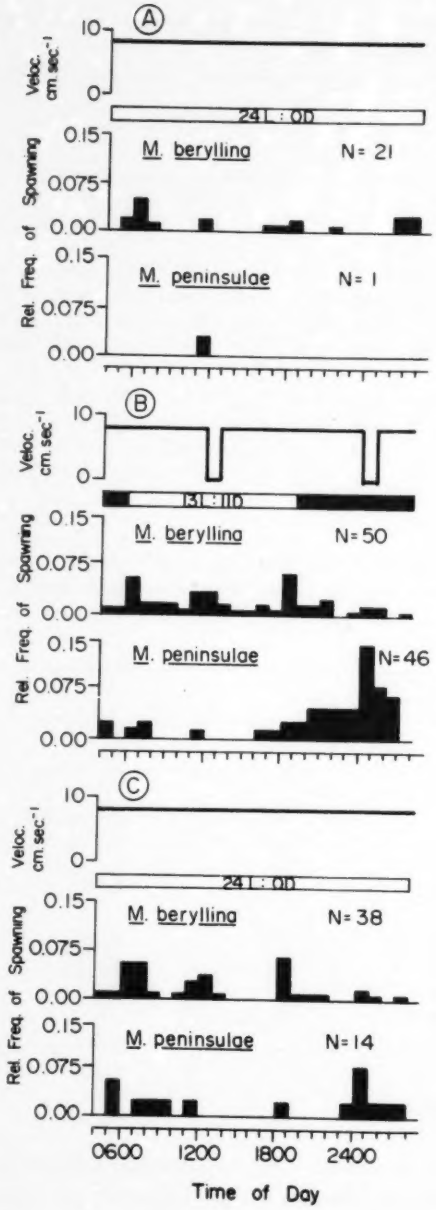

Fig. 3. Relative frequency of spawning by *M. beryllina* and *M. peninsulae* during respective periods of Experiment III: (A) constant conditions, (B) interrupted current velocity twice daily, 13L: 11D, (C) constant conditions. Refer to Table 1 for a synopsis of environmental spawning cues.

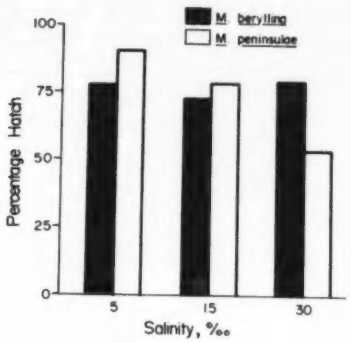

Fig. 4. Percentage hatching of *M. beryllina* and *M. peninsulae* eggs cultured at three salinities and 25 ± 1° C.

growth at 15‰. No mortalities occurred from 6 days after hatching until termination of the test on day 16 (Fig. 5a). *M. peninsulae* demonstrated a different trend; survival was best at 30‰, intermediate at 15‰ and lowest at 5‰ (Fig. 5b).

Growth data for each species are summarized in Table 3. Locations of significant differences in growth patterns at the three test salinities are identified in Table 4. Growth of *M. beryllina* (standard

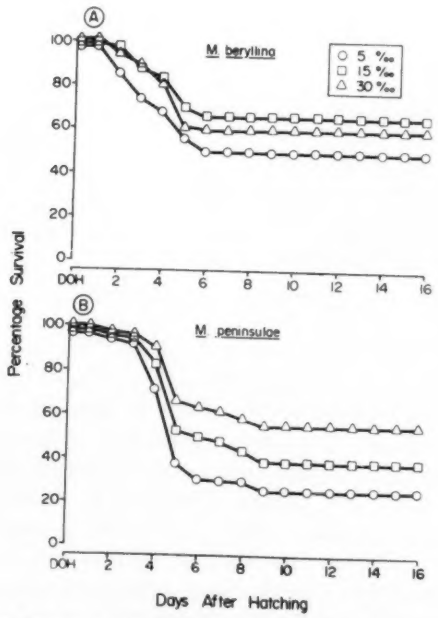

Fig. 5. Survival of larval *Menidia* cultured from the day of hatching (DOH) through 16 days old at three salinities.

Table 3. Summary data for growth of *M. beryllina* and *M. peninsulae* cultured at 5, 15 and 30‰. Reported values are means for samples of 9 to 34 individuals taken on the day of hatching (0) and 4, 8, 12 and 16 days after hatching.

	Age (days)														
	0			4			8			12			16		
Salinity ‰	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30
M. beryllina															
SL (mm)	3.32	3.33	3.40	3.84	3.84	3.64	4.49	4.56	4.51	6.34	6.40	6.15	8.15	8.66	8.29
TL (mm)	3.80	3.89	3.86	4.31	4.29	4.21	5.04	5.14	5.09	7.25	7.16	6.63	9.19	9.77	9.26
Wet wt. (mg)	0.15	0.16	0.15	0.19	0.16	0.17	0.38	0.43	0.46	1.71	1.56	1.31	3.83	5.23	4.10
Dry wt. (mg)	0.03	0.04	0.03	0.05	0.04	0.03	0.07	0.07	0.09	0.29	0.27	0.23	0.68	0.88	0.69
M. peninsulae															
SL (mm)	3.60	3.63	3.51	3.63	3.82	3.72	4.24	4.36	4.31	6.56	6.10	6.74	7.62	8.07	8.34
TL (mm)	3.91	3.96	3.84	3.93	4.16	4.04	4.64	4.79	4.66	7.31	6.78	7.57	8.42	9.02	9.29
Wet wt. (mg)	0.18	0.16	0.19	0.12	0.16	0.16	0.29	0.31	0.25	1.66	1.43	1.80	2.73	3.26	3.53
Dry wt. (mg)	0.03	0.04	0.04	0.02	0.04	0.04	0.06	0.06	0.06	0.28	0.19	0.31	0.45	0.57	0.61

Table 4. Summary of growth data for larval *M. beryllina* and *M. peninsulae* held at 5, 15 and 30‰ from the day of hatching (0) through 16 days old. One-way analysis of variance (ANOVA) tests were conducted for each age group. The Student-Neuman-Keuls (SNK) procedure was utilized to identify differences among salinities within each age group. Underscored means are similar: * $P<0.05$, ** $P<0.01$, NS – no significant difference for larvae cultured at each salinity.

	Age (days)				
	0	4	8	12	16
Menidia beryllina					
SL (mm)	NS	NS	NS	NS	$\underline{\bar{X}_5, \bar{X}_{30}}, \bar{X}_{15}$**
TL (mm)	NS	NS	NS	NS	$\underline{\bar{X}_5, \bar{X}_{30}}, \bar{X}_{15}$**
Wet Wt. (mg)	NS	NS	NS	NS	$\underline{\bar{X}_5, \bar{X}_{30}}, \bar{X}_{15}$**
Dry Wt. (mg)	NS	$\underline{\bar{X}_5, \bar{X}_{15}, \bar{X}_{30}}$*	NS	NS	$\underline{\bar{X}_5, \bar{X}_{30}}, \bar{X}_{15}$**
Menidia peninsulae					
SL (mm)	NS	NS	NS	NS	$\underline{\bar{X}_5, \bar{X}_{15}, \bar{X}_{30}}$*
TL (mm)	NS	NS	NS	NS	$\underline{\bar{X}_5, \bar{X}_{15}, \bar{X}_{30}}$*
Wet Wt. (mg)	NS	NS	NS	NS	NS
Dry Wt. (mg)	NS	$\underline{\bar{X}_5, \bar{X}_{15}, \bar{X}_{30}}$**	NS	$\underline{\bar{X}_5, \bar{X}_{30}}, \bar{X}_{15}$*	NS

and total length), and wet and dry weight) measured on day 16 was significantly greater ($P<0.01$) at 15‰ than at 5 or 30‰ (Table 4). Measurements on younger *M. beryllina* (DOH, 4, 8, 12 days old) revealed no obvious pattern for optimal growth at the three test salinities (Tables 3, 4). While no trend was apparent in the growth of *M. peninsulae*, in cases where significant differences did occur, larvae cultured at 30‰ were generally larger than those maintained at 5‰ (Table 4).

Discussion

Our laboratory data for spawning patterns in *M. beryllina* indicate a high frequency of spawning during day and night while *M. peninsulae* spawned primarily at night, suggesting that substantial overlap in reproductive activity is possible in nature. However, to gain an understanding of factors which could play a part in the observed reproductive isolation of each species (Johnson 1975, Echelle & Mosier 1982, Echelle et al. 1983) it is also

important to consider their occurrence along salinity and tidal gradients within estuaries.

A synopsis of data for the relative frequency of species occurrence in estuarine systems along the Texas coast is presented in Figure 6. Data were summarized from Echelle & Mosier (1982) and include only collections from locations where free movement of stocks along a salinity gradient was possible. Therefore, all data for species occurrence and abundance in reservoir systems were deleted from the analyses. *M.beryllina* showed a preference for intermediate salinities of 11 to 20‰ while *M. peninsulae* demonstrated a trend of increased abundance with increasing salinity (Fig. 6).

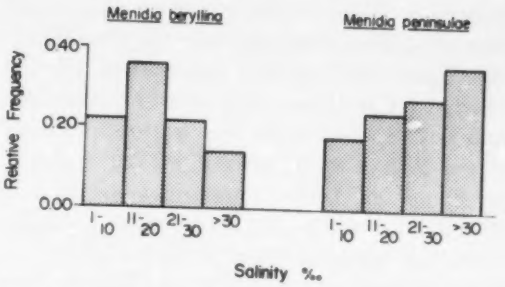

Fig. 6. Relative frequencies of occurrence of *M. beryllina* and *M. peninsulae* in riverine and estuarine environments of coastal Texas. From Echelle & Mosier (1982).

Thus several environmental and behavioral interactions resulting in reproductive isolation of each species seem possible. First, *M. beryllina* showed a much higher frequency of spawning than *M. peninsulae*, irrespective of environmental cues. Moreover, *M. beryllina* will spawn in the absence of environmental cues. Although *M. peninsulae* also reproduced in the absence of cues, the frequency of spawning was very low. It appears that *M. peninsulae* typically residing at intermediate to high salinities, must be presented a combination of 'tidal' and diel light cycle cues before high frequencies of reproductive activity and synchronized spawning will occur. In contrast, *M. beryllina*, generally occurring at low to intermediate salinities, showed a high frequency of spawning in the absence of cues and also in the presence of cues. So it appears that the two species, although demonstrating an overlap in estuarine distribution may spawn in response to different environmental signals. *M. peninsulae* spawns when some combination of interrupted current velocity (tidal) and diel light cycle cues are presented in concert, while *M. beryllina* appears to need no such synchronization of environmental signals and may respond to the singular influence of a diel light cycle. Indeed, Hubbs (1976) observed a diel rhythm in the gonadosomatic index of *Menidia audens* (conspecific with *M. beryllina*, Chernoff et al. 1981) from nontidal Lake Texoma. *M. beryllina* showed a peak in the percentage of ripe females between 0500 and 1000 h indicating that spawning generally occurred in midmorning. He reported that nearly all females produce egg clutches daily in June and July. Echelle (1983, personal communication) observed spawning *M. beryllina* in Lake Texoma during daytime.

Johnson (1975) reported a very low frequency of *M. beryllina* × *M. peninsulae* hybrids, two from Texas and one from Florida during an extensive survey of the biochemical systematics of *Menidia*. However, recent evidence provided by Echelle (1983, personal communication) suggests that *M. beryllina* × *M. peninsulae* forms may occur at a much higher frequency. A collection of 57 *Menidia* sp. from Pirates' Cove, Santa Rosa Island, Florida, a locality where very low and very high tidal current velocities occur in close proximity at intermediate salinities yielded 39 *M. peninsulae* (68%), 11 *M. beryllina* (19%) and 7 *M. beryllina* × *M. peninsulae* hybrids (12%). Similar localities in Texas produced a 2 to 3% occurrence of *M. beryllina* × *M. peninsulae* forms (Echelle & Mosier 1982).

It is interesting that the Pirates' Cove locale provides habitats where a weak tidal current (natural diel light cycle without an interrupted current velocity cue), and a strong tidal current (interrupted current velocity cue acting in concert with the natural diel light cycle) occur together. This locale has produced the highest percentage of *M. beryllina* × *M. peninsulae* forms observed in nature.

During this study, parental brood stocks of *M. beryllina*, collected from coastal Blackwater Bay, were maintained at salinities of 4 to 6‰ in the

laboratory. These adults produced eggs which were euryhaline at salinities from 5 to 30‰. Hatching ranged from 73 to 78%. Substantially different results for salinity tolerance of *M. beryllina* eggs from Lake Texoma stocks were reported by Hubbs et al. (1971). They observed a marked decline in percentage hatched at intermediate salinities of 20 and 25‰, with no hatching occurring at 33‰. Thus, it is apparent that eggs from stocks of *M. beryllina* which have resided in Lake Texoma since at least 1953 (Riggs & Bonn 1959) have a reduced tolerance to saline waters when compared to eggs from fish found in Blackwater Bay, an open estuarine environment where rapid fluctuations in salinity are common.

In this study, *M. peninsulae* eggs spawned by brood stocks collected from Santa Rosa Island, where salinities generally are from 20 to 28‰, tolerated a wide range of salinities. However, we observed an inverse relationship between incubation salinity and percentage hatching (Fig. 4). Considering the occurrence of *M. peninsulae* at intermediate to high salinities (Fig. 6; Johnson 1975, Echelle & Mosier 1982) the high percentage of hatching at a low salinity is surprising.

Moreover, we learned that *M. beryllina* larvae showed optimal survival and growth when cultured at an intermediate salinity of 15‰, a finding that is consistent with the salinity preference of adults. While the highest survival of *M. peninsulae* larvae occurred at 30‰, a trend similar to the salinity preference of adults, no such relationship was apparent in growth of larvae at 5, 15 or 30‰.

In summary, spawning patterns of *M. beryllina* and *M. peninsulae* in the laboratory suggest a possible mechanism for genetic isolation of the two species in nature. *M. beryllina* spawned in the absence of interrupted current velocity (tidal) or diel light cycle cues while *M. peninsulae* had to be presented with a combination of cues to elicit a high frequency of spawning. *M. beryllina* embryos were euryhaline. In contrast, *M. peninsulae* embryos had a significantly higher percentage of successful hatching at 5‰ than at 15 or 30‰ salinity. Survival and growth of larval *M. beryllina* at low, intermediate and high salinities in the laboratory followed the general trend of distribution for adults in the field. Survival of *M. peninsulae* larvae was highest at 30‰, a finding consistent with the occurrence of adults, however, no trend was apparent in the growth of larvae at low, intermediate and high salinities.

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